Spent Brewer's Yeast Extracts as a New Component of Functional Food

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Abstract

PODPORA B., ŚWIDERSKI F., SADOWSKA A., RAKOWSKA R., WASIAK-ZYS G. (2016): **Spent brewer's yeast extracts as a new component of functional food**. Czech J. Food Sci., 34: 554–563.

The use of yeast extracts as a natural and valuable additive ingredient intended for the production of functional food and dietary supplements were demonstrated. The chemical composition, amino acid analysis, determination of protein molecular weights, antioxidant properties, and sensory evaluation were carried out for two yeast extracts. It was found that the tested extracts are characterised by high essential amino acid content, exceeding the levels of reference protein developed by the FAO/WHO, and high antioxidant activity. Sensory characteristics of tested extracts may favourably influence the quality of the proposed functional foods and dietary supplements. The obtained results indicate that the tested extracts can be utilised as a source of free amino acids and peptides in the design of functional foods and dietary supplements.

Keywords: brewing by-products; flavour enhancers; natural food additives

In recent years we have observed a growing interest of the food industry in brewing by-products, such as spent brewer's yeast. Most of the spent brewer's yeast, a waste product, is used as a source of protein, B vitamins, and minerals in animal feed production. Spent brewer's yeast is not only a good source of inexpensive protein (45–60%), B vitamins, minerals, and other substances from the wort, but also it can be a good source of a number of valuable ingredients with pro-health properties such as β-glucans or mono- and oligosaccharides (YORK & INGRAM 1996; Ferreira et al. 2010; Jarmołowicz et al. 2013; Waszkiewicz-Robak 2013). Brewer's yeast is generally regarded as a safe microorganism, which contains several beneficial nutrients (MUSSATTO 2009; Waszkiewicz-Robak 2013). However, yeast consumption as a protein source for human nutrition is limited by the high level of nucleic acids present. Specifically, yeast contains 6-15% nucleic acids as compared to 2% in meat products. It is known that high intake of nucleic acids in the human diet can increase the uric acid levels in blood at physiological pH, which can result in hyperuricemia or the

deposition of uric acid in joint tissue. Research has shown that the safe level for nucleic acid intake for humans is 2 g/day, which amounts to 20 g yeast solids per day based on the fact that yeast contains 10% of nucleic acid (Schulz & Oslage 1976; Reed & Nagodavithana 1991). Increased amounts of yeast in the diet would require a decrease in the nucleic acid content of the yeast itself; it is possible to reduce this content up to 2% (Damodaran & Kinsella 2004; Trevelyan 2006).

A new direction in the application of spent brewer's yeast is the production and the use of some of its bioactive compounds in functional food, such as β-glucan, mono- and oligosaccharides, as well as extracts which can be obtained from spent brewer's yeast. Yeast extracts are widely used as natural flavour enhancers to replace glutamates and nucleotides in many processed foods. Improved taste results from the synergistic interaction of the 5-nucleotide with amino acids (especially glutamic acid) and peptides, which are contained in extracts. In the case of extracts obtained from spent brewer's yeast, the level of their addition to food products is defined by sensory prop-

erties and therefore, there is no risk associated with exceeding a safe level of nucleic acids (Ferreira et al. 2010; VIEIRA et al. 2013; PODPORA et al. 2015). Yeast extracts are produced by yeast cell wall degradation using endogenous or exogenous enzymes (Breddam & Beenfeld 1991; Choi & Chung 1998; Chae et al. 2001). The methods of extract preparation are divided into autolysis and hydrolysis. Autolysis with endogenous enzymes occurs in the yeast when the cell cycle is completed and the death phase is initiated. Enzymatic reactions occur spontaneously in the autolysis process, which is catalysed by endogenous yeast enzymes that are activated at the appropriate process conditions such as temperature and pH. The autolysis process has some disadvantages such as low extraction yield and difficulty in the solid-liquid separation process. In a modified autolysis process, referred to as plasmolysis, inorganic salts such as sodium chloride or non-polar organic solvents are often used to accelerate autolysis. Due to the high salt content, yeast extracts manufactured by plasmolysis may have limited uses (Belousova et al. 1995; Chae et al. 2001). Yeast extracts can also be prepared by acid hydrolysis or enzymatic hydrolysis, and the resulting products are known as 'yeast hydrolysates'. Despite a high production yield, acid hydrolysis is less attractive to manufacturers due to the relatively high salt content and high probability of the presence of carcinogenic compounds such as monochloropropanol and dichloropropanol. The enzymatic hydrolysis of yeast cells is more desirable as it produces extracts with a low salt content (Nagodawithana 1992; Podpora et al. 2015). A study done by PODPORA et al. (2015) shows that when the process of autolysis is properly conducted, it is possible to obtain autolysates with varying free amino acid content and peptides of different molecular weights tailored to the specific nutritional needs. This allows a broader use of autolysates in functional foods and dietary supplements.

Yeast extracts are valuable, natural flavourings which enhance the meat flavour in various types of food products and spice mixtures. They replace more widely used protein hydrolysates obtained by the acidic method, and more recently they have also replaced monosodium glutamate. There is a limited amount of literature on the use of yeast extracts in the production of functional foods as a source of amino acids and peptides (Podpora *et al.* 2015). The aim of this study was to evaluate the composition and functional properties of commer-

cial yeast extracts from spent brewer's yeast and to subsequently determine the possibility of their application not only as a flavouring or as a source of nutrients in microbiological growth media, but also as a component of functional food and dietary supplements. Consequently, if the high nutritional value of commercially obtained tested extracts is confirmed, this study may be valuable from both the scientific and the application point of view. In that case, the extracts would be able to perform a dual role – they would be functional components allowing for a health claim while simultaneously serving as flavouring agent or a source of nutrients in microbiological growth media.

MATERIAL AND METHODS

The study was conducted using two yeast extracts developed by Leiber GmbH, Bramsche, Germany. The extracts were in the form of a free flowing powder: extract A (Leiber-Viande B, LS; product code 44700P-059) is used as a flavour enhancer for food products and extract B (Leiber Fermentation; product code 44200P-101) is used as a microbial culture medium. Brewer's yeast was selected as the raw material for yeast extract production. The dry matter content of the raw material was 12-15%. In order to remove hop residues, sieving was carried out using vibrating sieves with a mesh diameter less than 1 mm. The raw material was transferred to vessels where temperature (55-60°C for extract A and 50-55°C for extract B) and oxygen (in the case of extract A) were adjusted via an automatic system. Papain was used to perform enzymatic hydrolysis. The process lasted 24 h with constant mixing. The obtained yeast hydrolysate was separated with centrifuges to remove the cell walls. The liquid fraction (yeast extract) was debittered and clarified, then evaporated and finally spray dried at 180°C/80°C, where 180°C is the air inlet temperature and 80°C is the air outlet temperature at the bottom of the tower. All other details of the production technology of the extracts (sieve opening, oxygenation, enzyme activity and commercial name, enzyme catalogue number and producer, amount of enzyme, centrifuging condition, details on column parameters used to clarify the extracts, debittering, and spray drying conditions) are protected by the Leiber Company.

Analytical methods. The analysis of the chemical composition of extracts was conducted as per the

method of AOAC 16th editon (1995). Dry matter and ash content were determined in the extracts using a gravimetric method. In addition, the presence of insoluble ash was determined using a 10% HCl solution. The content of nitrogen was analysed by the Kjeldahl method via utilisation of a conversion factor of 6.25 for nitrogen quantities of protein and nucleic acid content. The fat content was analysed using the Soxhlet method. Determination of the total sugar content was performed using the Lane-Eynon method in accordance with the Polish standard (PN-A-79011-5:1998 – Food concentrates - Methods of test – Determination of sugars).

The determination of the amino acid content in the tested extracts was preceded by acid hydrolysis in order to obtain free amino acids. After hydrolysis, the derivatisation using dansyl chloride was carried out, and then the amino acids were separated and determined using high performance liquid chromatography with UV/VIS detection (Stephens 1986). Alkaline hydrolysis was used for the determination of tryptophan and the exact tryptophan content was determined using HPLC with fluorescence detection (Bech-Andersen 1991).

The Chemical Score (CS), i.e. the ratio of the amount of amino acid in a yeast extract to the amount of the corresponding amino acid in standards multiplied by 100 (Block & Mitchell 1946), and the Essential Amino Acid Index (EAAI) were calculated based on the amino acid composition (OSER 1951). The standard of whole egg and those developed by the FAO/WHO (1991) have been adopted for use as the protein amino acid composition pattern. A standard developed by the FAO/WHO for children was used due to the higher value of individual amino acids included in this standard as compared to the adult standard. In the present work, concentrations of amino acids such as methionine and cysteine, and phenylalanine and tyrosine, are given together like in the literature. This is due to the fact that cysteine and tyrosine are formed from the essential amino acids methionine and phenylalanine, respectively. Therefore, these amino acids (cysteine and tyrosine) are known as conditionally essential amino acids.

The determination of the molecular weights of specific proteins was performed by mass spectrometry using a mass spectrometer equipped with a MALDI ion source type. Specifically, a MALDI-TOF Reflex IV mass spectrometer, with operation in linear mode (Bruker-Daltonics, Bremen, Germany) was used. The analysis was performed in the range of 500–5500, 2000–19 000, and 10 000–105 000 m/z.

Sinapic acid (SA) and α -cyano-4-hydroxycinnamic acid (HCCA) (dissolved in a 2:1 volume of acetonitrile in water to saturate the solution) were used as a matrix. Each sample was thoroughly mixed and centrifuged (10 000 RCF, 10 min, 4°C). Then, the supernatant was diluted five times with 0.1% TFA. A mixture of 0.5 ml of the prepared sample plus 0.5 ml of the matrix was loaded on the plate and then the plate was allowed to dry. After drying, the sample was introduced into the mass spectrometer ion source and ionised. One microgram of the sample was used for a single analysis. The nominal sensitivity of the spectrometer for the peptides of $1000-5000\ m/z$ was 1 fmol.

The antioxidant properties were determined by the spectrophotometric *in vitro* method using the synthetic ABTS radicals according to the method in Pellegrini *et al.* (2003).

The polyphenol content was measured by the Sin-GLETON and Rossi (1965) method using the Folin-Ciocalteu reagent.

Sensory method. Detailed sensory characteristics of the extract samples were performed by Quantitative Descriptive Analysis (QDA) in accordance with the regulatory procedure described in ISO 13299:2003 -Sensory analysis - Methodology - General guidance for establishing a sensory profile. To analyse the profile of the extracts, 18 quality parameters were selected - the smell: yeast, mushroom, bouillon, burnt, grain, sweet, sour, other; the taste: yeast, mushroom, bouillon, burnt, grain, sweet, sour, salty, bitter, stinging, other; and the overall quality of the extracts was rated. The sensory characteristics of the samples were evaluated by an 8-person team of evaluators who are qualified assessors and experts according to PN-EN ISO 8586:2014-03 -Sensory analysis - General guidelines for the selection, training and monitoring of selected assessors and experts of sensory evaluation, and who have methodological (theoretical and practical) training in the field of sensory methods. The sensory evaluation was performed in two independent replications. Ratings were performed at the Laboratory of Sensory Analysis of the accredited Laboratory of Food Evaluation and Health Diagnostics, which meets all the requirements specified in the standard BS EN ISO 8589:2010 - Sensory analysis. General guidance for the design of test rooms. The ANALSENS NT computerised support system of sensory analysis (CogITos, Sopot, Poland) was used for planning sessions with the rating scale method, the generation of random numbers for coding of samples, recording of individual results, and the pre-treatment of samples.

Statistical methods. The significance of differences in the sensory characteristics between the compared extracts was verified by the multivariate analysis of variance (ANOVA). This analysis was performed on individual sample results using the profiled method of sample evaluation in order to determine whether sample variation significantly affects differences in the intensity of individual quality trials and monitors the impact of the session and evaluators' team regarding the results of the evaluations. The significance of differences between means was verified in accordance with the NIR criterion. The significance of differences in chemical research of yeast extracts was verified using a one-way analysis of variance (ANOVA). The significance of differences between means was verified by the LSD (least significant difference) test. Results of the statistical analysis are presented in the table using appropriate letters of the alphabet which defined the homogeneous group.

RESULTS AND DISCUSSION

Chemical composition of yeast extracts. The basic composition of the tested yeast extracts is presented in Table 1. The obtained results indicate that the high protein content in both extracts was comparable, notably above 60%. This high protein content is due to the fact that the yeast interior is separated from the cell wall components. Therefore, a large amount of free amino acids (including glutamic acid that affects the flavour) remains in the solution while all of the non-protein components and parts of heavier proteins associated with the cell wall that were not autolysed are removed from the solution. Hence, the extracts were characterised by a low content of total sugars, a high proportion of ash and a low content of insoluble ash in the HCl reagent. Similar results were reported by Podpora et al. (2015) when the examined autolysates were obtained from spent brewer's yeast. In Sommer (1998), the obtained results regarding the chemical composition of yeast extracts derived from Saccharomyces cerevisiae differed significantly from those examined in the current study. The extracts from the studies by Sommer (1998) contained higher protein (73–75%), sugar (5%), and fat (5%) contents.

Analysis of the amino acid composition of extracts. The results of the amino acid composition of the tested extracts (Table 2) indicate that the proteins of the tested extracts were hydrolysed into free amino acids. The obtained high content of free amino acids in the tested extracts was due to the long action of lytic enzymes on the yeast proteins throughout the entire autolysis process, the aim of which was to increase degradation of the tested material. This resulted in a high concentration of glutamic acid in the final product, which exhibits strong flavour-enhancing properties in the food (Reed & Nagodawithana 1991; Sommer 1998; Stone 1998). The contents of free glutamic acid in extracts A and B were 3.84 and 2.07%, respectively. It was noteworthy that the tested extracts had high contents of relatively exogenous amino acids (amino acids that can be produced within the body, but must be supplied from food in states of increased demand; GAWĘCKI & HRYNIEWIECKI 2005) such as arginine, histidine, and serine, and endogenous amino acids such as proline and glycine, which are important in the creation of collagen in the body. The quantified amino acid content of yeast autolysates obtained in the present work (Table 2) is different from the amino acid composition of pure brewer's yeast, Saccharomyces cerevisiae (Sommer 1998). This is due to the fact that the autolysates investigated in the present work were not prepared from pure brewer's yeast but from the wort remaining in the brewing process. This suspension is rich in proteins, peptides, and free amino acids derived from barley malt and other additives employed in beer production. Consequently, the amino acid profile of the obtained extracts was characterised by a much higher concentration of amino acids than are normally present in yeast cells. In such a case the amino acid profile is thus dependent on the wort composition (Sommer 1998; Podpora et al. 2015). Importantly, the obtained results are consistent with data found in the literature (SOMMER 1998; SUHAJDA et al. 2000; PODPORA et. al. 2015).

Table 3 shows a comparison of the exogenous amino acid content in the tested yeast extracts with respect to

Table 1. The chemical composition of the tested yeast extracts (in %)

Yeast extracts	Dry weight	Fat	Protein content	Sugar	Ash	Ash insoluble in HCl
Extract A	95.20 ± 0.211	0.10 ± 0.030	62.50 ± 0.159	2.90 ± 0.011	9.50 ± 0.194	0.10 ± 0.017
Extract B	93.20 ± 0.174	0.20 ± 0.019	63.80 ± 0.092	2.90 ± 0.008	7.80 ± 0.058	0.08 ± 0.018

Table 2. Amino acid content in yeast extracts A and B

A : -1	Total (g/100 g)		Free amino a	Free amino acids (g/100 g)		Free amino acids (%)	
Amino acid	A	В	A	В	A	В	
Aspartic acid	6.18	5.79	2.23	2.36	36.08	40.76	
Threonine	2.68	2.59	1.57	1.44	58.58	55.60	
Serine	2.91	2.84	2.27	1.67	78.01	58.80	
Glutamic acid	8.86	9.05	3.84	2.07	43.34	22.87	
Glycine	2.91	2.94	1.53	1.01	52.58	34.35	
Alanine	4.44	4.18	3.76	3.09	84.68	73.92	
Cysteine	0.51	0.74	0.21	0.31	41.18	41.89	
Valine	3.55	3.44	2.52	1.98	70.99	57.56	
Methionine	0.90	0.90	0.74	0.62	82.22	68.89	
Isoleucine	2.90	2.80	2.17	1.65	74.83	58.93	
Leucine	4.10	4.09	3.52	2.98	85.85	72.86	
Tyrosine	1.81	2.17	1.41	1.35	77.90	62.21	
Phenylalanine	2.54	2.74	2.10	1.87	82.68	68.25	
Histidine	1.34	1.36	1.00	0.64	74.63	47.06	
Lysine	4.34	4.14	2.06	1.63	47.47	39.37	
Arginine	3.02	2.73	1.99	1.43	65.89	52.38	
Proline	2.81	3.79	1.78	2.00	63.35	52.77	
Tryptophan	0.71	0.78	0.58	0.50	81.69	64.10	
Total	56.51	57.07	35.28	28.60	62.43	50.11	

the exogenous amino acid content in protein standards of hen eggs, which is established by the FAO/WHO (1991). The obtained amino acid content in the tested extracts shows that they are a very valuable source of a number of amino acids such as isoleucine, lysine, valine, threonine, and phenylalanine + tyrosine. The contents of these amino acids, as compared to the reference protein developed by FAO/WHO, was higher in both examined extracts. However, lower values of these

amino acids were observed as compared to the whole egg protein reference. It is worth emphasising that the tested extracts are characterised by a considerable sum of exogenous amino acids of 365 mg/g of protein for extract A and 356 mg/g protein for extract B. These values are higher than the sum of the amino acids in the reference protein developed by FAO/WHO and much lower than the reference amino acid composition of whole egg protein. The integrated ratio of

Table 3. Comparison of the content of essential amino acids in the yeast extracts in relation to the content of amino acids in protein standards of whole egg and established by the FAO/WHO (1991)

	Exti	acts	Star	ndards	CS (wh	ole egg)	CS (FAC	D/WHO)
Amino acid		(m	g/g protein)		extr	acts	extr	acts
	A	В	whole egg	FAO/WHO	A	В	A	В
Isoleucine	44	41	54	28	81	76	157	146
Leucine	62	60	86	66	72	70	94	91
Lysine	66	60	70	58	94	86	114	103
Methionine + cysteine	21	24	57	25	37	42	84	96
Phenylalanine + tyrosine	66	72	93	63	71	77	105	114
Threonine	41	38	47	34	87	81	121	112
Tryptophan	11	11	17	11	65	65	100	100
Valine	54	50	66	35	82	76	154	143
Sum of exogenous amino acids	365	356	490	320				
EAAI					71	70	114	112

CS – chemical score; EAAI – essential amino acids index

essential amino acid index (EAAI), as an indicator of the maximum potential of the nutritional value of protein (Gawęcki & Hryniewicki 2005), reached values of 114 and 112 for extracts A and B, respectively, in relation to the reference protein developed by the FAO/WHO. The obtained results indicate that the tested extracts are a valuable source of amino acids that are important from a nutritional point of view. The high total exogenous amino acid content in extracts exceeded the levels of those present in the

standard developed by the FAO/WHO, which indicated that these extracts can be a valuable component of functional foods and dietary supplements in which the presence of free amino acids and low molecular weight peptides is required, i.e. supplements for athletes and food for particular nutritional uses (Boza *et al.* 2000; Manninen 2009).

Analysis of the molecular weight distribution of proteins, peptides, and amino acids. Figure 1 shows the results of the MALDI analysis for the tested

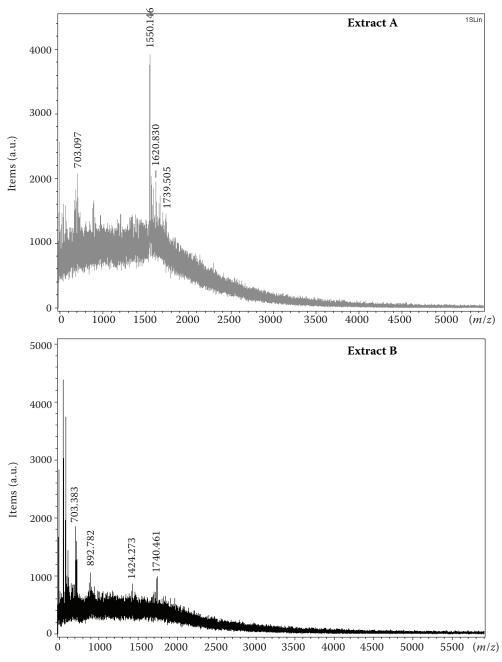


Figure 1. Analysis of the molecular weight distribution of protein, peptides, and amino acids of extract A and B by mass spectrometry with the MALDI ion source in the field of $500-5500 \ m/z$

extracts A and B in units of m/z (m/z is the ratio of the mass to the ion charge of the molecule; $1 m/z \approx 1 \text{kDa}$). For extract A, peaks with low intensities of 703 and $1550 \, m/z$ were observed, while no peaks were observed above $1800 \, \text{m/z}$, demonstrating the presence of high molecular weight compounds. Similarly, in the case of extract B, peaks of 703, 892.8, 1424.3, and $1740.5 \, m/z$ were observed. These peaks were characterised by a low intensity. In both cases there were no peaks that demonstrated the presence of high molecular weight compounds in the spectra greater than $1750 \, m/z$.

Additionally, the presence of high molecular weight compounds in the tested extracts was not revealed in the mass ranges of 2000–19 000 and 10 000–105 000 m/z. Accordingly, it can be assumed that both extracts contain only trace amounts of high molecular weight compounds, namely proteins. These results confirm the data reported by SOMMER (1998) and PODPORA et al. (2015), who described the content of oligopeptides, the weight of which was higher than 3000 Da by approximately 2-5% in extracts used for microbiological purposes. On the basis of the results obtained by the MALDI analysis and the amino acid composition, it can be said that both yeast extracts contain large amounts of free amino acids and only trace amounts of peptides, the molecular weight of which is higher than 1740 Da. The remaining peptides, with molecular weights significantly lower than the aforementioned values, prove that the examined protein extracts are almost completely hydrolysed in order to achieve very high levels of free amino acids and low molecular weight peptides.

Evaluation of antioxidant properties and content of polyphenolic compounds. The tested extracts are characterised by a very high antioxidant activity ranging from 461.5 mmol TEAC/100 mg to 506.9 mmol TEAC/100 mg (Table 4). These values are comparable with the antioxidant activity of tea (Pellegrini et al. 2003; Almajano et al. 2008; Dumarey et al. 2008). Noticeable high antioxidant activity is probably related to the high levels of soluble residues of polyphenolic compounds remaining after sieving, which was derived from the hops and barley used in the brewing process. The total content of polyphenolic compounds evaluated was 37.51 mg GAE/100 mg for

extract A and 48.73 mg GAE/100 mg for extract B. These results are consistent with the results obtained in the study of PODPORA *et al.* (2015).

Sensory characteristics of yeast extract. The sensory qualities of the tested yeast extracts, summarised in Table 5, were varied and dependent on the intensity and changing proportions of many flavour traits. The extract sensory profiles were mainly due to the presence of yeast, mushroom, bouillon, burnt flavours, and grain smell. The intensity of all evaluated quality parameters, except for the other smell and taste, changed significantly with increasing extract concentrations. Qualitative and quantitative changes in the intensity of the evaluated discriminants were much less dependent on the type of extract, despite some differences in the production method. While producing extract A, apart from the addition of enzymes (which contributed to the acceleration of the autolysis process and increased the degradation of proteins and peptides to amino acids), the process was carried out in the presence of oxygen. The oxygenation of the extract in the production process was employed to increase the intensity of flavour and colour, which could result from the formation of Maillard reaction products. Regardless of the differences in the methods of obtaining extracts, no significant differences in their sensory profile have been observed. Apparently, the notes of yeast, mushroom, burnt, bitter, and burning were marked with substantially variable intensity with increasing extract concentration in the sensory profile of the tested extracts. Significant changes also occurred in the grain smell and the bouillon taste and smell. The nature and quantity of changes in the sensory profile of the yeast extracts, intended as meat flavouring agents, were more dependent on their concentration rather than their type. It is worth mentioning that changes depending on the type of extract were more prominent at higher concentrations (1%). Further, the increasing extract concentration resulted in a decreased overall quality. Thus, it can be concluded that the tested extracts had a clear bouillon note justifying their usage in the production of meat flavour preparations or, on the other hand, as flavouring agents to replace previously used protein

Table 4. Antioxidant activity and the content of polyphenolic compounds in the tested yeast extracts

Yeast extracts	Antioxidant activity (mmol TEAC/100 mg)	Total content of polyphenolic compounds (mg GAE/100 mg)
Extract A	461.50 ± 1.860	37.510 ± 0.033
Extract B	506.90 ± 2.431	48.730 ± 0.033

Table 5. Evaluation and statistical significance of differences between samples of the intensity of quality of smell and taste as well as overall quality parameters

Sensory quality parameters		Extr	act A concentra	tion	Extract B concentration			
		0.1%	0.5%	1%	0.1%	0.5%	1%	
	yeast	1.99ª	3.34 ^b	4.24 ^c	1.98ª	3.95 ^{bc}	5.13 ^d	
	mushroom	1.58 ^a	$2.34^{\rm b}$	3.17 ^{cd}	1.33 ^a	2.89^{bc}	3.76^{d}	
	bouillon	1.39^{ab}	1.95^{bc}	$2.14^{ m cd}$	1.12 ^a	$2.02^{\rm c}$	2.66^{d}	
ell	burnt	1.38^{a}	2.54^{b}	3.22^{bc}	1.19 ^a	2.67^{b}	4.05^{c}	
Smell	grain	1.16^{a}	1.73^{b}	1.93 ^b	1.11 ^a	2.00^{b}	2.13^{b}	
	sweet	0.88^{a}	$1.44^{\rm b}$	1.62 ^b	0.78^{a}	1.76^{b}	1.83 ^b	
	sour	0.71 ^a	1.29^{ab}	1.42^{bc}	0.73^{a}	1.83 ^{bc}	1.98 ^c	
	other	0.01	0.18	0.16	0.06	0.13	0.22	
	yeast	2.17ª	3.66 ^b	4.46 ^b	2.01 ^a	3.54 ^b	4.37 ^b	
	mushroom	1.56 ^a	2.65^{bc}	3.34^{bc}	1.34^{a}	2.81^{b}	3.93^{c}	
	bouillon	1.34^{a}	2.06^{bc}	2.06 ^{bc}	1.34^{a}	1.9^{ab}	$2.54^{\rm c}$	
a)	burnt	1.83 ^a	3.31^{b}	3.84^{b}	1.39 ^a	3.08^{b}	4.96°	
Taste	grain	1.07^{a}	1.83^{abc}	$1.42^{ m abc}$	0.89^{a}	1.69 ^c	1.49^{bc}	
Η	sour	0.89^{a}	$1.44^{\rm b}$	1.92 ^b	0.84^{a}	1.99^{bc}	2.58^{c}	
	salty	0.49^{a}	$1.14^{\rm b}$	1.10^{b}	0.55^{a}	$1.21^{\rm b}$	1.29^{b}	
	stinging	1.61 ^a	2.78^{bc}	3.10^{bc}	1.53ª	2.29^{ab}	4.12^{c}	
	other	0.08	0.23	0.44	0.00	0.19	0.51	
Overall quality		4.54^{bc}	4.08 ^{ab}	3.63 ^{ab}	4.73°	3.89 ^{abc}	3.36 ^a	

 $^{^{}a-d}$ average values with different letters differ significantly among each other ($\alpha = 0.05$)

hydrolysates obtained by acid hydrolysis. The sensory characteristics of the two extracts indicate that their usage in functional foods and dietary supplements may have beneficial effects on the sensory quality.

There is little research on the sensory quality of yeast extracts in the literature. The Podpora *et al.* (2015) study has shown that autolysates obtained from spent brewer's yeast were characterised by a different sensory profile with a noticeably bitter aftertaste derived from the bitter substances extracted from hops. This bitter aftertaste changed favourably with the durational increase of the autolysis process and growth of the free amino acid content, particularly glutamic acid.

CONCLUSIONS

The tested yeast extracts obtained from the spent brewer's yeast using the autolysis method, assisted by the addition of proteolytic enzymes, are characterised by a high content of essential amino acids, which exceeds that of the reference protein developed by the FAO/WHO. The high content of free amino acids and high levels of peptides with low molecular weights indicated that the tested extracts may be use-

ful not only as flavourings, but also in applications as a source of free amino acids and peptides in the design of functional foods and dietary supplements wherein the level of their additive is limited by the sensory quality and level of nucleic acids.

The tested extracts have high antioxidant activities comparable to those of tea, and a high content of polyphenols, which could be affected by the composition of wort used in the beer production process.

The tested yeast extracts are characterised by a similar bouillon taste profile with a noticeable bitter aftertaste derived from the bitter substances, which is mainly due to the high degree of hydrolysis of proteins and the high content of free glutamic acid, which can come from beer. These extracts can be particularly useful in designing a new assortment of functional foods of the bouillon taste profile that is critically absent on the market.

References

Almajano M.P., Carbó R., Limenéz A.L., Gordon M.H. (2008): Antioxidant and antimicrobial activities of tea infusions. Food Chemistry, 108: 55–63.

- Bech-Andersen S. (1991): Determination of tryptophan with HPLC after alkaline hydrolysis in autoclave using α-methyl-tryptophan as internal standard. Acta Agriculturae Scandinavica, 41: 305–309.
- Belousova N.I., Gordienko S.V., Eroshin V.K. (1995): Influence of autolysis conditions on the properties of aminoacid mixtures produced by ethanol-assimilating yeast. Applied Biochemistry and Microbiology, 31: 391–395.
- Block R.J., Mitchell H.H. (1946): The correlation of the amino acid composition of proteins with their nutritive value. Nutrition Abstracts and Reviews, 16: 249–278.
- Boza J.J., Moennoz D., Vuichoud J., Jarret A.R., Gaudard-de-Weck D., Ballevre O. (2000): Protein hydrolysate vs free amino acid-based diets on the nutritional recovery of the starved rat. European Journal of Nutrition, 39: 237–243.
- Breddam K., Beenfeld T. (1991): Acceleration of yeast autolysis by chemical methods for production of intracellular enzymes. Applied Microbiology and Biotechnology, 35: 323–329.
- Chae H.J., Joo H., In M.J. (2001): Utilization of brewer's yeast cells for the production of food-grade yeast extract. Part 1: Effects of different enzymatic treatments on solid and protein recovery and flavor characteristics. Bioresource Technology, 76: 253–258.
- Choi S.J., Chung B.H. (1998): Simultaneous production of invertase and yeast extract from baker's yeast. Biotechnology and Bioengineering, 13: 308–311.
- Damodaran S., Kinsella J.E. (2004): The use of chaotropic salts for separation of ribonucleic acids and proteins from yeast nucleoproteins. Biotechnology and Bioengineering, 25: 761–770.
- Dumarey M., Nederkassel A.M., Deconinck E., Vander Heyden Y. (2008): Exploration of linear multivariate calibration techniques to predict the total antioxidant capacity of green tea from chromatographic fingerprints. Journal of Chromatography, 1192: 81–88.
- Extract A. Leiber-Viande B, LS; Product code 44700P-059. Available at www.leibergmbh.de/int/food/products/leiber-viande/ (accessed May 25, 2016).
- Extract B. Leiber Fermentation; Product code 44200P-101. Available at www.leibergmbh.de/int/fermentation/ products/leiber-fermentation-s/ (accessed May 25, 2016).
- FAO/WHO (1991): Protein quality evaluation. Report of the Joint FAO/WHO Expert Consultation. FAO Food and Nutrition Paper 51. Available at http://apps.who.int/iris/bitstream/10665/38133/1/9251030979_eng.pdf (accessed May 25, 2016).
- Ferreira I.M.P.L.V.O., Pinho O., Vieira E., Tavarela J.G. (2010): Brewer's *Saccharomyces* yeast biomass: characteristics and potential applications. Trends in Food Science and Technology, 21: 77–84.

- Gawęcki J., Hryniewiecki L. (2005): Białka. In: Gawęcki J. (ed.): Żywienie Człowieka. Warszaw, Polskie Wydawnictwo Naukowe: 204–219.
- Jarmołowicz S., Zakęś Z., Siwicki A., Terech-Majewska E., Kowalska A., Partyka K., Hopko M. (2013): Immunomodulatory effect of dietary brewer's yeast extract in Sander lucioperca juveniles against the challenge of Aeromonas salmonicida. Aquaculture International, 21: 939–945.
- Manninen A.H. (2009): Protein hydrolysates in sports nutrition. Nutrition and Metabolism, 6: 1–10.
- Mussatto S.I. (2009): Biotechnological potential of brewing industry by-products. Biotechnology for agro-industrial residues utilisation. London, Springer, 313–326.
- Nagodawithana T. (1992): Yeast-derived flavors and flavor enhancers and their probable mode of action. Food Technology, 46: 138–144.
- Oser B.L. (1951): Method for integrating essential amino acid content in the nutritional evaluation of protein. Journal of the American Dietetic Association, 27: 396–402.
- Pellegrini N., Serafini M., Colombi B., Del Rio D., Salvatore S., Bianchi M., Brighenti F. (2003): Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different *in vitro* assays. American Society of Nutritional Sciences, 6: 2812–2819.
- Podpora B., Świderski F., Sadowska A., Piotrowska A., Rakowska R. (2015): Spent brewer's yeast autolysates as a new and valuable component of functional food and dietary supplements. Journal of Food Processing and Technology, 6: 526–530.
- Reed G., Nagodawithana T.W. (1991): Food and feed yeast.
 In: Reed G., Nagodawithana T.W. (eds): Yeast Technology. 2nd Ed. New York, Van Nostrand Reinhold: 413–440.
- Schulz E., Oslage H.J. (1976): Composition and nutritive value of single-cell protein (SCP). Animal Feed Science Technology, 1: 9–24.
- Singleton V.L., Rossi J.A. (1965): Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture, 16: 144–158.
- Sommer R. (1998): Yeast extracts: production, properties and components. Food Austria, 50: 181–183.
- Stephens K. (1986): Amino acid analysis by dansylation: a revised method. Muncie, Ball State University: 1–43.
- Stone C.W. (1998): Yeast products in the feed industry: a practical guide for feed professionals. Cedar Rapids, Diamond V.
- Suhajda A., Hegdczki J., Janzso B., Pais I., Vereczkey G. (2000): Preparation of selenium yeasts I. Preparation of selenium-enriched *Saccharomyces cerevisiae*. Journal of Trace Elements in Medicine and Biology, 14: 43–47.
- Trevelyan W.E. (2006): Determination of uric acid precursors in dried yeast and other forms of single-cell pro-

tein. Journal of the Science of Food and Agriculture, 26: 1673–1680.

Vieira E., Brandao T., Ferreira I.M.P.L.V.O. (2013): Evaluation of brewer's spent yeast to produce flavour enhancer nucleotides: influence of serial repiiching. Journal of Agricultural and Food Chemistry, 61: 8724–8729.

Waszkiewicz-Robak B. (2013): Spent brewer's yeast and beta-glucans isolated from them as diet components modifying blood lipid metabolism disturbed by an atherogenic diet. In: Baezin R.V. (ed.): Lipid Metabolizm. Croatia, InTech Pub: 261–290.

York S.W., Ingram L.O. (1996): Ethanol production by recombinant *Escherichia coli* KO11 using crude yeast autolysate as a nutrient supplement. Biotechnology Letters, 18: 683–688.

 $\label{eq:Received:2015-08-26}$ Accepted after corrections: 2016-11-24

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